

### **13d Directed Evolution of Homing Endonuclease with Altered DNA Sequence Specificity**

*Zhilei Chen and Huimin Zhao*

Homing endonucleases are enzymes that catalyze DNA sequence specific double-strand breaks and can significantly stimulate homologous recombination at these breaks. These enzymes have great potential for applications such as gene correction in gene therapy or gene alteration in systems biology and metabolic engineering. However, the limited natural repertoire of target sequences of homing endonuclease severely hampers their applications, and the lack of an efficient selection method restricts the use of powerful directed evolution approaches for engineering of homing endonucleases with novel sequence specificity in vitro. Here we report the development of a highly sensitive selection method for the directed evolution of homing endonucleases. This system links the DNA cleavage event by homing endonuclease with the survival of an E. coli cell under specific conditions. Using I-SceI as a model homing endonuclease, we have demonstrated that cells with wild type I-SceI showed a high cell survival rate of 80-100 % in the presence of the original I-SceI recognition site, whereas cells without I-SceI showed a survival rate less than 0.003%. Currently, we are using this selection method in combination with our recently developed in vitro coevolution technique, to rapidly engineer I-SceI variants that can selectively cleave a target sequence found in a mutant transmembrane conductance regulator (CFTR) gene involved in cystic fibrosis. This mutant gene contains a deletion ( $\Delta$ F508) and is responsible for most of the cases of cystic fibrosis, which affects one in 2500 to one in 1600 Caucasians. The engineered I-SceI may be used to correct this gene in a gene therapy regime.